Modulation of pain-associated hyperexcitability at central synapses of capsaicin-sensitive nociceptors

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Modulation of pain-associated hyper-excitability at central synapses of capsaicin-sensitive nociceptors.

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TRPV1-expressing (capsaicin-sensitive) afferents correspond largely to peptidergic nociceptors, which play an important role in both acute pain and chronic hyper-sensitive pain states. Investigation of processes that can modulate function of their early central synapses in spinal dorsal horn could point the way to novel analgesics for chronic pain. We have developed a new method to quantify receptor-evoked Ca2+ fluorescence responses of ex vivo synaptic preparations and use it here to measure capsaicin-evoked responses in dorsal horn from control and pain state animals.
Synaptoneurosomes (re-sealed presynaptic and closely apposed postsynaptic compartments) were prepared from dorsal lumbar spinal cord of male Sprague-Dawley rats, under conditions designed to maintain functional integrity, and loaded with a no-wash Ca2+ fluorophore (Calcium 5). Capsaicin or other agents (including ionomycin as a positive control) were added in vitro and responses measured by fluorometric plate reader.
Responses to capsaicin showed concentration-dependent increases from 0.2-10 microM, were 5-6 fold greater in dorsal than in ventral horn and were largely reversed by the TRPV1 antagonist AMG9810 or presynaptically acting tetanus toxin. In addition the responses were inhibited by antagonists of AMPA- or NMDA-type glutamate receptors, consistent with glutamatergic transmission from capsaicin-activated presynaptic terminals. Agents selective for several distinct subtypes of GluN2 subunit showed differential ability to inhibit capsaicin responses.
We further explored the effects of endogenous analgesic mechanisms. In vitro addition of mu (and to a lesser extent delta) opioids strongly attenuated capsaicin responses. In a model of chronic inflammatory pain (intraplantar Complete Freund's Adjuvant), ex vivo responsiveness to capsaicin was increased in a manner completely reversed by NMDA receptor antagonists. This inflammation-induced hypersensitivity at TRPV1 afferent central synapses was strongly attenuated by prior in vivo administration of the TRPM8 agonist, icilin (200 microM topical to hindpaws, 15 min).
These observations reveal quantifiable actions of established or novel analgesic targets impacting on central synapses of TRPV1-expressing nociceptors.